IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:
Chatterjee, D.K.
Appl. No. 09/558,421
Filed: April 26, 2000
For: **Mutant DNA Polymerases and Uses Thereof**

Art Unit: 1652
Examiner: Rao, M.
Atty. Docket: 0942.3600003/RWE/BJD

Declaration of Roger Lasken

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

I, Roger Lasken, do hereby declare and say:

1. THAT, I, Roger Lasken, hold the degree of Ph.D. A recent copy of my Curriculum Vitae, accurately listing my scientific credentials and work experience, is attached hereto as Exhibit A.

2. THAT, since March 15, 1998, I have been employed by Molecular Staging. Prior to my current position, from 1990 to 1997, I was employed by Life Technologies, Inc. (LTI) (and now Invitrogen Corporation)[1], the assignee of the above-captioned application, in the capacity of Principal Scientist. *See* Exhibit A.

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[1]Life Technologies, Inc. merged with Invitrogen Corporation on September 12, 2000, with Invitrogen Corporation being the surviving entity.

3. THAT, during my employment by LTI (and now Invitrogen Corporation), I worked under the supervision of Dr. Deb K. Chatterjee on a project involving the cloning, expression, and characterization of wild-type and mutant DNA polymerases.

4. THAT, I have reviewed my laboratory notebooks detailing my work on the project. Based on these laboratory notebook records and my recollection, the following activities involving my work, and relating to the DNA polymerase project, took place during the period from about October 16, 1994, until about September 8, 1995.

On or about October 18, 1994, I performed an experiment relating to the optimal conditions for the use of thermostable polymerases. In this experiment varying amounts of *Taq* polymerase were added to a DNA template to saturate the 3' end in a polymerase assay. The experiment suggested that *Taq* polymerase binding to DNA exhibits an equilibrium effect, and provided information regarding sequencing conditions with wild-type *Taq* polymerase. This experiment was recorded on pages 65-66 of notebook 3902. A copy thereof is attached as Exhibit 1.

On or about October 19, 1994, I performed an experiment investigating the optimal conditions for the use of thermostable polymerases in long PCR. In this experiment I labeled primers for long PCR with ^{32}P to optimize the *Tfl* polymerase concentration in long PCR reactions using mixtures of *Tfl* and Vent® polymerases. This experiment was used to provide additional information regarding sequencing conditions with *Tfl* polymerase. This experiment was recorded on page 67 of notebook 3902. A copy thereof is attached as Exhibit 2 .

On or about October 24, 1994, I began an experiment investigating the use of *Tfl* polymerase in long PCR reactions. A purpose of this experiment was to optimize the concentrations of *Tfl* polymerase in a mixture of *Tfl* and Vent® polymerases, for use in long PCR reactions, and to provide additional information regarding sequencing conditions with *Tfl* polymerase. This experiment was recorded on page 67 of notebook 3902. A copy thereof is attached as Exhibit 3.

On or about October 24, 1994, Roger Lasken continued an experiment on the use of *Tfl* polymerase in long PCR reactions. In this experiment I ran the samples from the reactions performed on October 24, 1994 on an ethidium bromide stained 8% agarose gel. This experiment was recorded on pages 67-69 of notebook 3902. A copy thereof is attached as Exhibit 4.

On or about October 25, 1994, I performed an experiment comparing the apparent molecular weights of a number of thermostable polymerases. In this experiment I ran an SDS-PAGE gel of eight thermostable DNA polymerases: *Tfl* (epicenter), *Tfl* (MBR), *Tth* (MBR), r*Tth* (Perkin Elmer), sequitherem (epicenter), Vent® (NEB), DeepVent® (NEB), and *Taq* EKBT1. This experiment was recorded on pages 72-73 of notebook 3902. A copy thereof is attached as Exhibit 5 .

On or about October 26, 1994, I continued my experiments on the use of *Tfl* polymerase in long PCR reactions. In this experiment I ran samples from reactions run on October 24, 1994, on an SDS page gel to determine the amount of degradation of the radioactive product. The gel

was exposed to a phosphoimager plate overnight. This experiment was recorded on pages 70-71 of notebook 3902. A copy thereof is attached as Exhibit 6.

On or about October 27, 1994, I continued my experiments on the use of *Tfl* polymerase in long PCR reactions. In this experiment I analyzed the data from the autoradiograph exposed overnight, and noted only slight degradation of product after 36 cycles. This experiment was recorded on pages 70-71 of notebook 3902. A copy thereof is attached as Exhibit 7.

On or about October 28, 1994, I made a 2.5 unit/μl stock of recombinant *Taq* (of EKBT1) by 1:1 dilution of a 5 unit/μl stock. This activity was recorded on page 74 of notebook 3902. A copy thereof is attached as Exhibit 8.

On or about November 1, 1994, I prepared additional experiments on the use of *Tfl* polymerase in long PCR reactions. I prepared labeled primers for a *Tfl*/Vent® primer degradation assay. Primers were prepared with αS and ribo ends. This activity was recorded on page 75 of notebook 3902. A copy thereof is attached as Exhibit 9.

On or about November 2, 1994, I continued my experiments on the use of *Tfl* polymerase in long PCR reactions. In this experiment I performed primer degradation assays using mixtures of *Tfl* and Vent® polymerases. This experiment was recorded on page 76 of notebook 3902. A copy thereof is attached as Exhibit 10.

On or about November 2, 1994, I performed an experiment investigating the optimal conditions for use of *Tne* polymerase in DNA sequencing. I set up sequencing reactions with *Tne* polymerase in Vent® and Cheng buffers. The reactions were analyzed by SDS PAGE, and exposed to a phosphoimager plate. The experiment showed that reactions did not sequence information when performed in Cheng buffer. This experiment was recorded on pages 77-78 of notebook 3902. A copy thereof is attached as Exhibit 11.

On or about November 4, 1994, I began experiments comparing the 3' to 5' exonuclease activity of *Tne* polymerase. I performed primer degradation assays on *Tne*, Vent®, and DeepVent® polymerases as controls in Cheng, Vent® and Klentaq buffers. I exposed phosphoimager plate overnight. This experiment was recorded on pages 80-81 of notebook 3902. A copy thereof is attached as Exhibit 12.

On or about November 5, 1994, I developed the autoradiograph image from the plate exposed on November 4, 1994, analyzed the data and prepared graphs depicting the data. The experiment showed that the turnover of *Tne* polymerase was approximately 2 times lower than both DeepVent® and Vent® polymerase. This experiment was recorded on pages 82-83 of notebook 3902. A copy thereof is attached as Exhibit 13.

On or about November 7-9, 1994, I performed an experiment to investigate the effect of buffer conditions on the 5' to 3' exonuclease activities of a number of polymerases. In this experiment I performed turnover experiments using Vent®, DeepVent®, and *Tne* polymerases in Cheng, Klentaq and Vent® buffer. The experiment showed that the buffer effect on turnover

was minimal. This experiment was recorded on pages 84-85 of notebook 3902. A copy thereof is attached as Exhibit 14.

On or about November 10, 1994, I further analyzed data from the experiment performed on November 9, 1994. This activity was recorded on pages 86-87 of notebook 3902. A copy thereof is attached as Exhibit 15.

On or about November 15, 1994, I performed a quality control experiment on recombinant *Taq* polymerase (r*Taq*) stocks for use as controls in additional experiments on the use of thermostable polymerases in sequencing and PCR. In this quality control experiment I determined unit activity concentration. This experiment was recorded on pages 90-91 of notebook 3902. A copy thereof is attached as Exhibit 16.

On or about November 30, 1994, I prepared EKBT1 r*Taq* dilutions (10 u/μl) for use by other employees of LTI in product scale-up development. This activity was recorded on pages 82-87 and 90-92 of notebook 3902. A copy thereof is attached as Exhibit 17.

On or about December 8, 1994, I began an experiment to study conditions for the use of *Taq* and Vent® polymerases in long PCR reactions. In this experiment I performed PCR reactions using mixtures of *Taq* and DeepVent® polymerases with no Mn^{2+} or dNTP bias. I received plates of cells that contained the pUC19 vector which would serve as the template for the PCR assays and picked 20 white colonies and 2 blue colonies. I inoculated them into LB

media with ampicillin, and grew them overnight. This experiment was recorded on page 93 of notebook 3902. A copy thereof is attached as Exhibit 18.

On or about December 9, 1994, I continued the experiment on conditions for *Taq* polymerase long PCR reactions by preparing the template for the long PCR experiments. I performed minipreps on the cultures grown overnight on December 8, 1994, and analyzed them by restriction mapping. This experiment was recorded on page 93 of notebook 3902. A copy thereof is attached as Exhibit 19.

Soon after December 10, 1994, I began an experiment to determine the heat stable polymerase activity of recombinantly expressed *Tfl*. I obtained seven clones containing the *Tfl* polymerase gene from Ayoub Rashtchian, another LTI employee, inoculated the cells into LB and induced expression. Cultures were centrifuged and the cell pellets were stored at -70°C overnight. This experiment was recorded on page 94 of notebook 3902. A copy thereof is attached as Exhibit 20.

On or about December 14, 1994, I continued my experiment to determine the heat stable polymerase activity of recombinantly expressed *Tfl* polymerase. I lysed and heat-treated host cell clones containing a recombinant *Tfl* gene, and assayed the lysates for polymerase activity. All clones were shown to contain heat stable polymerase activity. This experiment was recorded on page 95 of notebook 3902. A copy thereof is attached as Exhibit 21.

On or about December 15, 1994, I continued my experiment to determine the heat stable polymerase activity of recombinantly expressed *Tfl* polymerase. I analyzed the results from the heat stable polymerase assay performed on December 14, 1994, and prepared graphs. Additionally, I labeled primers for a further experiment to determine whether different polymerases can incorporate thymidine into a new DNA strand using a uracil containing template. These experiments were recorded on pages 97-99 of notebook 3902. A copy thereof is attached as Exhibit 22.

On or about December 16, 1994, I continued my experiment to determine whether different polymerases can incorporate thymidine into a new DNA strand using a uracil containing template. I performed assays for thymidine incorporation using a uracil containing template with Ultima, *Taq*, *Tne*, *Tfl*, *Tth*, Vent®, DeepVent®, Pfu and DTOK polymerases. Gels were run and exposed to a phosphoimager plate overnight. This experiment was recorded on pages 100-101 of notebook 3902. A copy thereof is attached as Exhibit 23.

On or about December 17, 1994, I continued my experiment to determine whether different polymerases can incorporate thymidine into a new DNA strand using a uracil containing template by analyzing data from the thymidine incorporation assays conducted December 15, 1994, and December 16, 1994. The experiment was not completed. This experiment was recorded on pages 102-103 of notebook 3902. A copy thereof is attached as Exhibit 24.

On or about January 11, 1995, I performed an experiment to determine the molecular weight of r*Taq* and native *Taq* polymerases by SDS polyacrylamide gel electrophoresis (SDS-

PAGE). This experiment was recorded on page 104 of notebook 3902. A copy thereof is attached as Exhibit 25.

On or about January 12, 1995, I prepared more template to be used in PCR experiments using various DNA polymerase to investigate fidelity of amplification using *Taq* or mixtures of *Taq* + DeepVent®. pUC19 clones were prepared for use as template. Additionally, I began restriction mapping the plasmids to confirm the identity of the clones. This experiment was recorded on page 106 of notebook 3902. A copy thereof is attached as Exhibit 26.

On or about January 13, 1995, I continued restriction mapping the pUC clones that I began on January 12, 1995. This activity was recorded on page 107 of notebook 3902. A copy thereof is attached as Exhibit 27.

On or about January 16, 1995, I outlined ideas and conditions for assays to measure the ability of a polymerase to incorporate thymidine into a new DNA strand from a uracil containing template. Primers were radiolabeled for these assays. This experiment was recorded on pages 108-109 of notebook 3902. A copy thereof is attached as Exhibit 28.

On or about January 17, 1995, I continued my experiment to measure the ability of a polymerase to incorporate thymidine into a new DNA strand from a uracil containing template. I performed the thymidine incorporation assays using recombinant *Taq* and Vent® polymerases. This activity was recorded on page 110 of notebook 3902. A copy thereof is attached as Exhibit 29.

On or about January 18, 1995, I continued my experiment to measure the ability of a polymerase to incorporate thymidine into a new DNA strand from a uracil containing template. I performed thymidine incorporation assays using SEQUENASE. This experiment was recorded on page 111 of notebook 3902. A copy thereof is attached as Exhibit 30.

On or about January 19, 1995, I analyzed data from the thymidine incorporation assays performed on January 17-18, 1995. This analysis was recorded on page 112 of notebook 3902. A copy thereof is attached as Exhibit 31.

On or about January 27, 1995, I began preparing template for various polymerase experiments, including the fidelity assays. I also prepared pUC clones to be used in PCR fidelity assays. This experiment was recorded on page 114 of notebook 3902. A copy thereof is attached as Exhibit 32.

On or about January 30, 1995, I digested the pUC clones purified on January 27, 1995, with restriction enzymes to confirm their identity. This experiment was recorded on page 115 of notebook 3902. A copy thereof is attached as Exhibit 33.

On or about January 31, 1995, I performed experiments to investigate the fidelity of recombinant *Taq* polymerase in long PCR reactions. In this experiment I performed PCR fidelity assays using recombinant *Taq* with and without Vent® polymerase in the presence of various concentrations of Mn^{++}. Reaction products were run on an agarose gel, and the results analyzed.

New dilutions of recombinant *Taq* polymerase were prepared. This experiment was recorded on pages 116-119 of notebook 3902. A copy thereof is attached as Exhibit 34.

On or about February 1, 1995, I performed a quality control experiment to aid in the investigation of the use of *Taq* polymerase. In this experiment I determined the pipette accuracy in order to optimize *Taq* polymerase storage pipetting. This experiment was recorded on page 120 of notebook 3902. A copy thereof is attached as Exhibit 35.

On or about February 2, 1995, I performed an experiment to confirm the identity of mutants from an experiment conducted on January 31, 1995, using restriction mapping. This experiment was recorded on pages 123-124 of notebook 3902. A copy thereof is attached as Exhibit 36.

On or about February 3, 1995, I performed an experiment to investigate the stability of recombinant *Taq* polymerase in various storage conditions. In this experiment I prepared recombinant *Taq* solutions with various buffers, and measured the polymerase activities of these solutions to serve as a baseline for future stability studies. This experiment was recorded on pages 121-122 of notebook 3902. A copy thereof is attached as Exhibit 37.

On or about February 10, 1995, I performed an experiment to optimize assay conditions for the primer degradation assays using Vent® polymerase. This experiment was recorded on pages 126-127 of notebook 3902. A copy thereof is attached as Exhibit 38.

On or about February 13, 1995, I continued my investigations of the optimal conditions for the primer degradation assays by running a 16% SDS-PAGE gel on the samples from February 10, 1995. This experiment was recorded on page 128 of notebook 3902. A copy thereof is attached as Exhibit 39.

On or about February 14, 1995, I continued my experiment to investigate the optimal conditions for the primer degradation assays by running additional gels and developing the phosphoimage from the experiments performed on February 10, 1995. I observed that increasing ionic strength in the buffer decreases the rate of degradation of the primers. This experiment was recorded on page 129 of notebook 3902. A copy thereof is attached as Exhibit 40.

On or about February 15, 1995, I continued my experiment to investigate the optimal conditions for the primer degradation assays by determining the effects of potassium chloride concentration on the assays when using *Taq* and *Tne* polymerases. This experiment was recorded on pages 130-131 of notebook 3902. A copy thereof is attached as Exhibit 41.

On or about February 16, 1995, I conducted an experiment to prepare a radioactive DNA ladder for use in later experiments. This experiment was recorded on page 132 of notebook 3902. A copy thereof is attached as Exhibit 42.

On or about February 17, 1995, I continued my experiment to investigate the optimal conditions for the primer degradation assays using recombinant *Taq* and *Tne* polymerases by analyzing the data from the assays performed on February 15, 1995, and found that potassium

chloride inhibits extension from the primed M13 template. This analysis was recorded on page 134 of notebook 3902. A copy thereof is attached as Exhibit 43.

On or about February 20, 1995, I performed an experiment to prepare reagents for use in the primer degradation assays by radiolabeling primers to be used in the those assays. This experiment was recorded on page 136 of notebook 3902. A copy thereof is attached as Exhibit 44.

On or about February 21, 1995, I performed an experiment to determine the extent of primer degradation under PCR conditions with different concentrations of KCl. This experiment was recorded on page 137 of notebook 3902. A copy thereof is attached as Exhibit 45.

On or about February 23, 1995, I began an experiment to ascertain the properties of various thermostable polymerases in applications such as sequencing, mutagenesis or PCR In this experiment I performed mismatch extension assays on *Taq*, *Tfl*, *Tne*, Vent®, DeepVent®, and Pfu polymerases. This experiment was recorded on page 138 of notebook 3902. A copy thereof is attached as Exhibit 46.

On or about February 24, 1995, I continued my experiment to ascertain the properties of various thermostable polymerases in applications such as sequencing, mutagenesis or PCR by analyzing data from the primer degradation assays performed on February 21, 1995. Additionally, I ran mismatch extension assays on *Taq*, *Tfl*, *Tne*, Vent®, DeepVent®, and *Pfu*

polymerases. This experiment was recorded on page 139-141 of notebook 3902. A copy thereof is attached as Exhibit 47.

On or about February 27, 1995, I continued my experiment to ascertain the properties of various thermostable polymerases in applications such as sequencing, mutagenesis or PCR by conducting mismatch repair assays on *Taq*, *Tfl*, *Tne*, Vent®, DeepVent® and *Pfu* polymerases. This experiment was recorded on pages 142-143 of notebook 3902. A copy thereof is attached as Exhibit 48.

On or about March 1, 1995, I performed an experiment to determine the effects of potassium acetate on the exonuclease activity of *Tne* and *Taq* polymerases. This experiment was recorded on pages 146-147 of notebook 3902. A copy thereof is attached as Exhibit 49.

On or about March 3, 1995, I performed an experiment to determine the effect of enzyme concentration on primer extension activity. These experiments were recorded on page 148-149 of notebook 3902. A copy thereof is attached as Exhibit 50.

On or about March 4, 1995, I analyzed the data from the *Tne* primer extension experiments performed on March 3, 1995. This analysis was recorded on page 150-151 of notebook 3902. A copy thereof is attached as Exhibit 51.

On or about March 9-13, 1995, I performed experiments to determine the stability of a *Taq* polymerase sample after 33 days of storage in PCR mix (original sample prepared February

2, 1995). This experiment was recorded on pages 152-153 of notebook 3902. A copy thereof is attached as Exhibit 52.

On or about March 15, 1995, I performed experiments to determine the optimal conditions for PCR reactions with *Taq* polymerase. In this experiment I noted that a 1/600 dilution of *Taq* polymerase should be used in PCR reactions between 20 and 40 minutes after diluting. This experiment was recorded on pages 155-156 of notebook 3902. A copy thereof is attached as Exhibit 53.

On or about March 20, 1995, I prepared tetracycline stocks and tetracycline plates for use in growing cells for *Tfl* polymerase expression and purification. This experiment was recorded on page 157 of notebook 3902. A copy thereof is attached as Exhibit 54.

On or about March 21, 1995, I conducted an experiment to begin the process of purifying recombinant *Tfl* polymerase by inoculating cells containing the *Tfl* polymerase gene to amp and tet plates. This experiment was recorded on page 157 of notebook 3902. A copy thereof is attached as Exhibit 55.

On or about March 22, 1995, I continued the experiment to purify recombinant *Tfl* polymerase by inoculating the *Tfl* clones to liquid media. Cells were grown and expression was induced. Following induction, cells were grown for an additional15 hours. This experiment was recorded on pages 157-158 of notebook 3902. A copy thereof is attached as Exhibit 56.

On or about March 23, 1995, I continued the experiment to purify recombinant *Tfl* polymerase. The cells grown on March 22, 1995, were lysed and the lysate was heat treated. Only low levels of polymerase activity were detected in the lysate after heat treatment, indicating the presence of only low levels of *Tfl* DNA polymerase. This experiment was recorded on pages 158-159 of notebook 3902. A copy thereof is attached as Exhibit 57.

On or about March 24, 1995, I prepared a new PEI stock solutions to be used in the purification of *Tfl* polymerase. This experiment was recorded on page 160 of notebook 3902. A copy thereof is attached as Exhibit 58.

On or about March 26-27, 1995, I began a new experiment to express and purify recombinant *Tfl* polymerase. I prepared new LB media and ampicillin stocks, into which the *Tf*106 clone was inoculated and grown. Expression was induced, and cells were grown for an additional hour. Cells were harvested and frozen at -70° C. This experiment was recorded on page 161 of notebook 3902. A copy thereof is attached as Exhibit 59.

On or about March 28, 1995, I continued my experiment to express and purify recombinant *Tfl* polymerase by preparing new buffers. This experiment was recorded on page 162 of notebook 3902. A copy thereof is attached as Exhibit 60.

On or about March 29, 1995, I continued the experiment directed to the isolation of recombinant *Tfl* polymerase. I lysed the cells which were grown on March 26, 1995. The lysate

was heat treated and PEI and ammonium sulfate precipitations were performed. This experiment was recorded on page 163 of notebook 3902. A copy thereof is attached as Exhibit 61.

On or about March 30, 1995, I continued the experiment to purify *Tfl* polymerase. In this experiment I performed polymerase assays on the supernatants from the ammonium sulfate precipitation performed on March 29, 1995. This activity was recorded on page 164-165 of notebook 3902. A copy thereof is attached as Exhibit 62.

On or about March 31, 1995, I continued the experiment to purify *Tfl* polymerase. I poured a Sephacryl 200 column for further use in *Tfl* isolation. This activity was recorded on page 166 of notebook 3902. A copy thereof is attached as Exhibit 63.

On or about April 4, 1995, I performed an experiment to test the stability of a sample of *Taq* polymerase which had been stored since February 3, 1995. This experiment was recorded on page 167-169 of notebook 3902. A copy thereof is attached as Exhibit 64.

On or about April 5, 1995, I began an experiment to purify *Tfl* polymerase by harvesting a new batch of recombinant *Tfl* polymerase-containing cells. The cells were lysed and heat treated. The heat stable polymerase activity was determined after heat treatment. This experiment was recorded on page 170 of notebook 3902. A copy thereof is attached as Exhibit 65.

On or about April 7, 1995, I continued the experiment to purify the *Tfl* polymerase by culturing additional cells containing *Tfl* polymerase. Cells were grown, induced, harvested, lysed and heat treated. Sodium chloride was added to the lysate and PEI and ammonium sulfate precipitations were performed. This experiment was recorded on pages 171-172 of notebook 3902. A copy thereof is attached as Exhibit 66.

On or about April 8, 1995, I continued the experiment to purify the *Tfl* polymerase by performing ammonium sulphate precipitation experiments. Since I was unable to get the ammonium sulfate pellet to sediment; the purification experiment was not continued. This experiment was recorded on page 173 of notebook 3902. A copy thereof is attached as Exhibit 67.

On or about April 11, 1995, I performed an experiment to investigate the stability of *Taq* polymerase. A sample of *Taq* polymerase that had been stored at room temperature for 29 days was assayed for polymerase activity. This activity was recorded on pages 174-175 of notebook 3902. A copy thereof is attached as Exhibit 68.

On or about April 13, 1995, I began a new experiment to purify *Tfl* polymerase by culturing cells containing the *Tfl* polymerase gene. Cells were harvested and lysed. Sodium chloride was added to the lysate. This activity was recorded on page 176 of notebook 3902. A copy thereof is attached as Exhibit 69.

On or about April 17, 1995, I continued the experiment to purify *Tfl* polymerase by performing PEI and ammonium sulfate precipitations on the lysates generated on April 13, 1995. This experiment was recorded on page 117 of notebook 3902. A copy thereof is attached as Exhibit 70.

On or about April 18, 1995, I continued the experiment to purify *Tfl* polymerase by applying a *Tfl* lysate to a Sephacryl 200 column and obtaining column fractions. Unit concentration assays were performed on the column fractions. This experiment was recorded on pages 178 and 180-181 of notebook 3902. A copy thereof is attached as Exhibit 71.

On or about April 19, 1995, I continued the experiment to purify *Tfl* polymerase by applying fractions isolated from the S200 column to a Blue Sepharose column. This experiment was recorded on pages 182 of notebook 3902. A copy thereof is attached as Exhibit 72.

On or about April 20, 1995, I continued the experiment to purify the *Tfl* polymerase by dialyzing fractions obtained from the Blue Sepharose column. The dialysate was then applied to a heparin column. This experimental activity was recorded on page 183 and 185 of notebook 3902. A copy thereof is attached as Exhibit 73.

On or about April 21, 1995, I continued the experiment to purify the *Tfl* polymerase by performing polymerase activity assays on the fractions from the heparin column. This experimental activity was recorded on pages 187-189 of notebook 3902. A copy thereof is attached as Exhibit 74.

On or about April 23, 1995, I prepared new tetracycline stock solutions and media. Media was quality controlled. This experimental activity was recorded on page 190 of notebook 3902. A copy thereof is attached as Exhibit 75.

On or about April 24, 1995, I regenerated the Blue sepharose, heparin and S200 columns used to purify the *Tfl* polymerase for future use. This experimental activity was recorded on page 191 of notebook 3902. A copy thereof is attached as Exhibit 76.

On or about April 25, 1995, I continued the experiment to purify the *Tfl* polymerase by performing SDS-PAGE on column fractions obtained from a previous experiment. This experimental activity was recorded on page 192 of notebook 3902. A copy thereof is attached as Exhibit 77.

On or about April 26, 1995, I continued the experiment to purify the *Tfl* polymerase by pooling column fractions obtained from a previous experiment and dialyzed them overnight. This activity was recorded on page 1 of notebook 3903. A copy thereof is attached as Exhibit 78.

On or about April 27, 1995, I continued the experiment to purify the *Tfl* polymerase by removing the samples from dialysis and running them on a heparin column to resolve two remaining peaks. This experimental activity was recorded on pages 2-3 of notebook 3903. A copy thereof is attached as Exhibit 79.

On or about April 28, 1995, I began an experiment to characterize purified *Tfl* polymerase fractions by performing SDS-PAGE on the fractions from the heparin column. This experimental activity was recorded on pages 4-5 of notebook 3903. A copy thereof is attached as Exhibit 80.

On or about April 29, 1995, I prepared the *Tfl* fractions for storage by preparing new *Taq* polymerase storage buffers, and pooling and dialyzing the *Tfl* polymerase fractions. This experimental activity was recorded on page 6 of notebook 3903. A copy thereof is attached as Exhibit 81.

On or about April 30, 1995, I stored the newly purified *Tfl* polymerase by mixing the dialyzed fractions with storage buffer and stored them at -20° C. This experimental activity was recorded on page 6 of notebook 3903. A copy thereof is attached as Exhibit 82.

On or about May 2, 1995, I performed an experiment to characterize the purified *Tfl* polymerase by performing unit activity concentration assays. This experimental activity was recorded on pages 7-8 of notebook 3903. A copy thereof is attached as Exhibit 83.

On or about May 3, 1995, I performed a further experiment to characterize the newly purified *Tfl* polymerase. I assayed the newly purified *Tfl* polymerase for endonuclease activity. This experimental activity was recorded on pages 9-10 of notebook 3903. A copy thereof is attached as Exhibit 84.

Between about May 3, 1995, and about May 9, 1995, I prepared dilutions of *Taq* polymerase for PCR functional assays. This experimental activity was recorded on page 11 of notebook 3903. A copy thereof is attached as Exhibit 85.

On or about May 9, 1995, I prepared for experiments comparing the properties of *Tfl* and *Tne* polymerases by labeling oligonucleotides for use in the fidelity assays. I also outlined conditions for assaying fidelity of polymerases with and without 3' exonuclease activity. Additionally, I performed unit activity concentration assays on *Tne* and *Tfl* polymerases. This activity was recorded on pages 12-13 of notebook 3903. A copy thereof is attached as Exhibit 86.

On or about May 10, 1995, I conducted an experiment to compare the properties of *Tne* and *Tfl* polymerases. I performed 3' exonuclease assays on *Tne* and *Tfl* polymerases in a number of buffer conditions. This experimental activity was recorded on pages 14-15 of notebook 3903. A copy thereof is attached as Exhibit 87.

On or about May 12, 1995, I conducted an experiment to continue characterizing the newly purified *Tfl* polymerase by assaying for 3' exonuclease activity. I noted that there was no 3' exonuclease activity detected. I also changed the *Tfl* storage conditions from *Taq* storage conditions to Epicenter *Tfl* polymerase conditions. This experimental activity was recorded on pages 16-19 of notebook 3903. A copy thereof is attached as Exhibit 88.

On or about May 16, 1995, I conducted an experiment to continue characterizing the newly purified *Tfl* polymerase. I performed polymerase assays on mixtures of *Tfl* and Vent® polymerases at various buffer conditions. This experimental activity is recorded on pages 20-21 of notebook 3903. A copy thereof is attached as Exhibit 89.

On or about May 17, 1995, Elizabeth Flynn or I conducted a unit assay on the *Tne* polymerase preparation from May 7, 1995. This experimental activity was recorded on page 22 of notebook 3903. A copy thereof is attached as Exhibit 90.

On or about May 18, 1995, I conducted an experiment to characterize fractions from the S200 column by assaying for polymerase activity. This experimental activity was recorded on pages 24-25 of notebook 3903. A copy thereof is attached as Exhibit 91.

On or about May 19, 1995, I conducted an experiment to continue characterizing the *Tne* polymerase by performing unit concentration assays on the fractions from the S200 column. This activity was recorded on page 25 of notebook 3903. A copy thereof is attached as Exhibit 92.

On or about May 22, 1995, I conducted an experiment to continue characterizing the properties of *Tfl* polymerase by performing 3' exonuclease activity assays on a mixture of *Tfl* and Vent® polymerases. This experimental activity was recorded on page 26 of notebook 3903. A copy thereof is attached as Exhibit 93.

On or about May 23, 1995, I conducted an experiment to continue characterizing the properties of *Tfl* polymerase by performing 3' exonuclease activity assays on a mixture of *Tfl* and Vent® polymerases. This experimental activity was recorded on pages 27-28 of notebook 3903. A copy thereof is attached as Exhibit 94.

On or about May 24, 1995, I analyzed data from the 3' exonuclease assays on mixtures of *Tfl* and Vent® polymerases. I also performed unit concentration assays on *Tfl*/Vent® polymerase mixtures prepared by Nin Guan, another LTI employee. Additionally, I performed long PCR experiments to measure the turnover of mixtures of *Tfl* and Vent® polymerases. This activity was recorded on pages 29-31 of notebook 3903. A copy thereof is attached as Exhibit 95.

On or about May 25, 1995, I performed unit concentration assays 1.1x r*Taq*, old r*Taq* (1.1x), new r*Taq* (1.1x) and BM2X polymerase mixtures. This experimental activity is recorded on pages 34-35 of notebook 3903. A copy thereof is attached as Exhibit 96.

On or about May 26, 1995, I analyzed the data collected in the unit activity concentration assays performed on May 25, 1995. This analysis is recorded on page 36 of notebook 3903. A copy thereof is attached as Exhibit 97.

On or about May 30, 1995, I performed unit activity concentration assays to determine the stability of 1.1x *Taq* polymerase stored at room temperature in reaction mixture. This

experimental activity was recorded on pages 37-39 of notebook 3903. A copy thereof is attached as Exhibit 98.

On or about May 31, 1995, Carolyn Combs and I performed experiments directed to determining the stability of thermostable polymerases. In this experiment they assayed the stability of *Tfl* and Vent® polymerases at 68° C over 20 minutes. This experimental activity was recorded on pages 40-42 of notebook 3903. A copy thereof is attached as Exhibit 99.

On or about May 31, 1995, I prepared new 5 unit/μl dilutions of r*Taq* polymerase, lot EKBT1. This experimental activity is recorded on page 43 of notebook 3903. A copy thereof is attached as Exhibit 100.

On or about June 2, 1995, I further characterized the *Tfl* polymerase by performing primer degradation assays using mixtures of *Tfl* and Vent® polymerase, and saw no apparent endonuclease or 5' exonuclease activity in the *Tfl* samples. This activity was recorded on pages 44-46 of notebook 3903. A copy thereof is attached as Exhibit 101.

On or about June 5, 1995, Carolyn Combs and I performed an experiment to assay mixtures of *Tfl* and Vent® polymerases for 3' exonuclease activity using the primer degradation assay. This experimental activity was recorded on pages 1-2 of notebook 4092. A copy thereof is attached as Exhibit 102.

On or about June 6, 1995, Carolyn Combs and I performed an experiment to assay mixtures of *Tfl* and Vent® polymerases for 3' exonuclease activity using the primer degradation assay. Additionally they determined the mobility of a full length ^{32}P labeled 33-mer on a PEI plate. This experimental activity was recorded on pages 3-4 and 7-8 of notebook 4092. A copy thereof is attached as Exhibit 103.

On or about June 7, 1995, Carolyn Combs and I performed an experiment to measure the 3' exonuclease activity in mixtures of *Tfl* and Vent® polymerases in 3 different buffer conditions to establish a baseline for the stability studies. This experimental activity was recorded on pages 9-10 of notebook 4092. A copy thereof is attached as Exhibit 104.

On or about June 9, 1995, I performed unit assays on Nin Guan's mixtures of *Tfl* and Vent® polymerases using various dilutions. Additionally I attempted to optimize the assay's signal to noise ratio and linearity. This experimental activity was recorded on pages 48-49 of notebook 3903. A copy thereof is attached as Exhibit 105.

On or about June 12, 1995, Carolyn Combs and I performed a quality control analysis on a new batch of PEI plates. Plates will be used for the *Tfl*/Vent® polymerase turnover experiments. This experimental activity was recorded on page 13 of notebook 4092. A copy thereof is attached as Exhibit 106.

On or about June 13, 1995, I performed an experiment to further characterize *Tfl* polymerase. I repeated exonuclease assays on *Tfl* polymerase using new primers. This

experimental activity is recorded on pages 50-51 of notebook 3903. A copy thereof is attached as Exhibit 107.

On or about June 13, 1995, Carolyn Combs and I performed an experiment to continue their characterization of *Tfl* polymerase. In this experiment they assayed both the turnover and incorporation rates of a mixture of *Tfl* and Vent® polymerases at varying concentrations of *Tfl* polymerase. This experimental activity was recorded on pages 17-18 of notebook 4092. A copy thereof is attached as Exhibit 108.

On or about June 14, 1995, Carolyn Combs and I further analyzed the data from the incorporation experiments performed on June 13, 1995. Additionally, they more accurately determined the turnover and incorporation rates of mixtures of *Tfl* and Vent® polymerases for use in the *Tfl*/Vent® stability studies. This activity is recorded on page 20-23 of notebook 4092. A copy thereof is attached as Exhibit 109.

On or about June 15, 1995, Carolyn Combs and I performed an experiment to continue the characterization of *Tfl* polymerase. They analyzed data from experiments performed on June 14, 1995. This activity is recorded on page 24 of notebook 4092. A copy thereof is attached as Exhibit 110.

On or about June 19, 1995, Carolyn Combs and I performed a further experiment to analyze turnover data from the experiments performed on June 13, 1995. Additionally, they determined the optimal running time for the PEI plates to minimize background when detecting

^{32}P-dATP. This experimental activity was recorded on page 19 of notebook 4092. A copy thereof is attached as Exhibit 111.

On or about June 20, 1995, Carolyn Combs and I analyzed data from the optimization experiments performed on June 19, 1995, and determined that errors occurred during the experiments. This activity was recorded on page 30 of notebook 4092. A copy thereof is attached as Exhibit 112.

On or about June 21, 1995, Carolyn Combs and I performed primer degradation assays on mixtures of *Tfl* and Vent® polymerases, as well as Vent® polymerase alone. This experimental activity was recorded on pages 33-36 of notebook 4092. A copy thereof is attached as Exhibit 113.

On or about June 23, 1995, I began experiments directed at the optimization of the M13 PCR system, which would be used in subsequent experiments on thermostable polymerases and their uses. On this date I performed experiments using *Tne* and *Taq* polymerases in the M13 PCR system at varying reaction conditions in an attempt to optimize the reaction buffer conditions. This activity was recorded on pages 40-41 of notebook 4092. A copy thereof is attached as Exhibit 114.

On or about June 24, 1995, Carolyn Combs and I further analyzed data and wrote conclusions based on the incorporation and turnover assays performed on June 14, 1995, and June 19, 1995. They determined that in order to optimally resolve the dATP/dADP peak from

the dAMP peak, the LiCl solvent should be run to the top of the PEI plate. This activity was recorded on pages 25, 31 and 42 of notebook 4092. A copy thereof is attached as Exhibit 115.

On or about June 26, 1995, Carolyn Combs and I performed experiments to optimize the M13 PCR system. In these experiments she diluted primers for use in the M13 PCR experiments to working concentrations, and ran agarose gels of the reaction products. Additionally the annealing temperature, *Taq* concentration, cycle number, and primer concentrations was optimized for the M13 PCR system. This experimental activity was recorded on pages 43-45 and 47-51 of notebook 4092. A copy thereof is attached as Exhibit 116.

On or about June 28, 1995, I performed an experiment to determine the stability of *Taq* polymerase when stored in reaction mix. Samples were analyzed which had been stored at 4° C, -20° C and -70° C for 0-5 months. This experimental activity was recorded on page 52 of notebook 3903. A copy thereof is attached as Exhibit 117.

On or about June 29, 1995, I analyzed data from the *Taq* stability assays performed on June 28, 1995. This activity was recorded on page 53 of notebook 3903. A copy thereof is attached as Exhibit 118.

On or about June 29, 1995, Carolyn Combs and I performed an experiment to continue the characterization of *Tfl* polymerase. They performed 3' exonuclease turnover assays on mixtures of *Tfl* and Vent® polymerases as well as Epicenter *Tfl*/Vent® polymerase mixture. Additionally, they optimized primer and enzyme concentrations in the M13 PCR system, and

prepared Mp19 ssDNA as a substrate for the 3' exonuclease activity assays. This experimental activity was recorded on pages 53-54 and 58-63 of notebook 4092. A copy thereof is attached as Exhibit 119.

On or about June 30, 1995, I performed an experiment continuing my analysis of *Taq* stability when stored in reaction buffer. This activity was recorded on page 53 of notebook 3903. A copy thereof is attached as Exhibit 120.

On or about June 30, 1995, Carolyn Combs, working under supervision by me, continued the characterization of *Tfl* polymerase by analyzing data from the turnover experiments performed on June 29, 1995. This analysis was recorded on page 55 of notebook 4092. A copy thereof is attached as Exhibit 121.

On or about June 30, 1995, Carolyn Combs and I performed 3' exonuclease turnover assays using mixtures of *Tfl* and Vent® polymerases with Mp 19 ssDNA as a template. This activity was recorded on page 64 of notebook 4092. A copy thereof is attached as Exhibit 122.

On or about July 1, 1995, Carolyn Combs and I continued the characterization of the *Tfl* polymerase by analyzing data from the 3' exonuclease turnover assays performed on June 30, 1995. This activity was recorded on pages 66-67 of notebook 4092. A copy thereof is attached as Exhibit 123.

On or about July 5, 1995, Carolyn Combs and I performed a further experiment to optimize primer concentration, annealing temperature, and template concentration for the M13 PCR system. This activity was recorded on pages 69-70 of notebook 4092. A copy thereof is attached as Exhibit 124.

On or about July 6, 1995, Carolyn Combs and I optimized annealing temperature, template concentration, and enzyme concentration in the M13 PCR system using *Tne* and *Taq* polymerases. This activity was recorded on pages 71-76 of notebook 4092. A copy thereof is attached to as Exhibit 125.

On or about July 7, 1995, Carolyn Combs and I continued the experiment to further optimize the enzyme concentrations used in the M13 PCR system. This activity was recorded on page 77 of notebook 4092. A copy thereof is attached as Exhibit 126.

On or about July 10, 1995, Carolyn Combs and I performed reactions comparing the activities of old and new samples of *Tne* polymerase in the M13 PCR system. This activity was recorded on page 78 of notebook 4092. A copy thereof is attached as Exhibit 127.

On or about July 11, 1995, Carolyn Combs, working under supervision by me, continued characterization of the *Tfl* polymerase by generating additional graphs from the turnover experiment data obtained on June 29, 1995. This activity was recorded on page 57 of notebook 4092. A copy thereof is attached as Exhibit 128.

On or about July 11, 1995, Carolyn Combs and I further optimized the M13 PCR assay system by optimizing enzyme concentration when Cheng buffer is used. This activity was recorded on page 81 of notebook 4092. A copy thereof is attached as Exhibit 129.

On or about July 12, 1995, Carolyn Combs and I began experiments characterizing the properties of *Tne* polymerase. On this date they performed an experiment to determine if *Tne* polymerase can produce the 3 smallest M13 PCR fragments in the M13 PCR assay system. This activity was recorded on page 84 of notebook 4092. A copy thereof is attached as Exhibit 130.

On or about July 13, 1995, Carolyn Combs and I continued the characterization of *Tfl* polymerase. In this experiment they performed 3' exonuclease turnover assays using *Tfl*/Vent® polymerase mixtures with Mp19 ssDNA as the template. This activity was recorded on pages 87-88 of notebook 4092. A copy thereof is attached as Exhibit 131.

On or about July 14, 1995, Carolyn Combs and I performed reactions under various conditions to determine which component of Cheng buffer is responsible for a smear seen on gels of PCR reaction products when the reactions were performed using *Tne* polymerase. They also performed other experiments to rule out other causes of the smear. Additionally they ran gels of the optimization reactions performed on July 11, 1995 (*see* Exhibit 129). This activity was recorded on pages 82-83, 85-86, 90-92 and 94-97 of notebook 4092. A copy thereof is attached as Exhibit 132.

On or about July 15, 1995, Carolyn Combs and I prepared new stop solutions. This activity was recorded on page 79 of notebook 4092. A copy thereof is attached as Exhibit 133.

On or about July 18, 1995, Carolyn Combs and I began purification of *Tne* polymerase using a purification strategy similar to that utilized in the *Taq* polymerase purification. New buffers were made, cells were spun, lysed and heat treated. PEI and ammonium sulfate precipitations were performed on the lysate and activities were measured. Additionally, a new strategy for large scale purification of *Tne* polymerase was outlined. New cells were spun, lysed and heat treated, NaCl was added and a PEI precipitation was performed. This activity was recorded on page 100-103 and 105 of notebook 4092. A copy thereof is attached as Exhibit 134.

On or about July 19, 1995, Carolyn Combs and I continued the purification of *Tne* polymerase. In this experiment they performed an ammonium sulfate precipitation on the fractions from the PEI precipitation of the large scale *Tne* polymerase preparation. Blue sepharose and S200 columns were equilibrated, and ammonium sulfate fractions were run on an S200 column. This activity was recorded on page 106-107 of notebook 4092. A copy thereof is attached as Exhibit 135.

On or about July 20, 1995, Carolyn Combs and I performed experiments in support of their efforts to purify *Tne* polymerase. They performed unit concentration assays on the fractions from the S200 column, and the fractions were run on a Blue sepharose column. This activity was recorded on pages 108-109 of notebook 4092. A copy thereof is attached as Exhibit 136.

On or about July 21,1995, Carolyn Combs and I performed unit activity concentration assays on the fractions of the large scale *Tne* polymerase preparation that were run on a Blue Sepharose column (*see* Exhibit 136). The fractions from that column were then dialyzed overnight. This activity was recorded on pages 109-111 of notebook 4092. A copy thereof is attached as Exhibit 137.

On or about July 22, 1995, Carolyn Combs and I preformed unit concentration assays on old and newly purified samples of *Tne* polymerase. A heparin column was loaded with dialysate from July 21, 1995. Fractions from the heparin column were pooled, dialyzed and unit activity concentration assays were performed. This activity was recorded on pages 112 and 114-117 of notebook 4092. A copy thereof is attached as Exhibit 138.

On or about July 23, 1995, Carolyn Combs and I used the purified *Tne* polymerase fractions from the Blue sepharose and heparin column to see if they produced a characteristic smear when used in PCR reactions. This activity was recorded on pages 118-120 of notebook 4092. A copy thereof is attached as Exhibit 139.

On or about July 27, 1995, Carolyn Combs and I began comparisons of *Tne* and Tal polymerases in the polymerase chain reaction. In this experiment they optimized PCR conditions using *Tne* and *Taq* polymerases. This activity was recorded on pages 122-127 of notebook 4092. A copy thereof is attached as Exhibit 140.

On or about July 28, 1995, I continued comparing *Tne* and *Taq* polymerase by assaying *Taq*, *Tne* and Vent® polymerases for ability to extend and incorporate thymidine into a newly synthesized DNA strand from a uracil containing template. This activity was recorded on pages 54 and 56-57 of notebook 3903. A copy thereof is attached as Exhibit 141.

On or about July 28, 1995, Carolyn Combs and I further investigated the use of *Tne* polymerase on PCR reactions. They determined the effect of annealing temperature on the formation of the smear in PCR reactions with *Tne* polymerase. This activity was recorded on pages 128-129 of notebook 4092. A copy thereof is attached as Exhibit 142.

On or about July 31, 1995, I performed unit activity concentration assays on 1.1x *Taq* polymerase to be used in future experiments. This activity was recorded on page 60 of notebook 3903. A copy thereof is attached as Exhibit 143.

On or about July 31, 1995, Carolyn Combs and I compared hot start and cold start PCR using *Tne* polymerase. Additionally they determined that the addition of genomic DNA to the PCR reaction had no effect on the formation of the smear. This activity was recorded on pages 130-132 of notebook 4092. A copy thereof is attached as Exhibit 144.

On or about August 1, 1995, Carolyn Combs and I determined the effect of low Mg^{++} concentration on short PCR with *Tne* polymerase. This activity was recorded on pages 133 and 135 of notebook 4092. A copy thereof is attached as Exhibit 145.

On or about August 2, 1995, Carolyn Combs and I analyzed results of the PCR experiments performed on August 1, 1995. Additionally the effect of Mg^{++} concentration on long PCR using *Tne* and *Taq* polymerases was investigated. Oligonucleotides were also radiolabeled for use in future experiments. This activity was recorded on pages 134, 136-138 and 140 of notebook 4092. A copy thereof is attached as Exhibit 146.

On or about August 3, 1995, Carolyn Combs and I continued experiments in support of their comparisons of *Tne* and *Taq* polymerase. In this experiment they determined the ability of *Taq* polymerase to extend from short primers at varying *Taq* concentrations. This activity was recorded on pages 142-143 and 145 in notebook 4092. A copy thereof is attached as Exhibit 147.

On or about August 4, 1995, Carolyn Combs and I compared the fidelity of *Taq* and *Tne* polymerases using primers of varying lengths. This activity was recorded on pages 146-147 of notebook 4092. A copy thereof is attached as Exhibit 148.

On or about August 7, 1995, Carolyn Combs and I analyzed the data from the Mg^{++} titration experiments performed on August 2, 1995, and the fidelity experiments performed on August 4, 1995. This activity was recorded on pages 139 and 148-149 of notebook 4092. A copy thereof is attached as Exhibit 149.

On or about August 8, 1995, I radiolabeled primers for 3' exonuclease assays. This activity was recorded on page 61 of notebook 3903. A copy thereof is attached as Exhibit 150.

On or about August 8, 1995, Carolyn Combs, working under supervision by me, compared the time course for primer extension of *Taq* and *Tne* polymerases using varying concentrations of enzymes. This activity was recorded on pages 152-153 of notebook 4092. A copy thereof is attached as Exhibit 151.

On or about August 9, 1995, I began characterization of a 3' exonuclease mutant of the Klenow fragment of *E. coli* polymerase I, obtained from Dr. Catherine Joyce at Yale University (New Haven, CT), by performing 3' exonuclease activity assays to determine the amount of residual activity in the mutant. Additionally I assayed the polymerase activity of r*Taq* polymerase at various dTTP concentrations to determine what the optimal dTTP concentration is for maximum synthesis rate. This activity was recorded on pages 62-66 of notebook 3903. A copy thereof is attached as Exhibit 152.

On or about August 9, 1995, Carolyn Combs, working under supervision by me, continued the comparison of *Taq* and *Tne* polymerases. In this experiment she performed processivity assays on *Taq*, *Tne* and UlTma® polymerases by extension of a 33-mer primer annealed to M13 ssDNA. This activity was recorded on pages 155-156 of notebook 4092. A copy thereof is attached as Exhibit 153.

On or about August 10, 1995, Carolyn Combs, working under supervision by me, optimized PCR conditions when using a 16-mer oligonucleotide primer with *Tne* and *Taq* polymerases. This activity was recorded on pages 158-162 of notebook 4092. A copy thereof is attached as Exhibit 154.

On or about August 11, 1995, I continued the comparisons of *Tne* and *Taq* polymerase. On this date I performed processivity assays on *Tne*, *Taq* and Ultima polymerases similar to those performed on August 9, 1995, but at lower polymerase concentrations. This activity was recorded on page 164 of notebook 4092. A copy thereof is attached as Exhibit 155.

On or about August 14, 1995, I analyzed the data from the exonuclease experiments performed on August 9, 1995, comparing the activities of *Taq* and *Tne* polymerases. Additionally I determined the K_m of *Taq* polymerase for dTTP in the polymerase assay. This activity was recorded on page 63 of notebook 3903. A copy thereof is attached as Exhibit 156.

On or about August 15, 1995, I continued my experiments into the stability of thermostable polymerases. In this experiment I optimized the dATP, dGTP and dCTP concentrations in the reaction buffer used for long term storage of *Taq* polymerase. Optimization was based on polymerase activity. This activity was recorded on pages 68-71 of notebook 3903. A copy thereof is attached as Exhibit 157.

On or about August 15, 1995, Carolyn Combs, working under supervision by me, treated *Tne* polymerase with DNase to determine if genomic DNA contamination is the cause of the smear in PCR reactions using *Tne*. This activity was recorded on pages 166-168 of notebook 4092. A copy thereof is attached as Exhibit 158.

On or about August 16, 1995, I repeated unit activity concentration assays on 1.1x *Taq* polymerase. This activity was recorded on page 72 of notebook 3903. A copy thereof is attached as Exhibit 159.

On or about August 16, 1995, Carolyn Combs and I analyzed data from DNase treatment experiments performed on August 15, 1995. This activity was recorded on pages 169-174 of notebook 4092. A copy thereof is attached as Exhibit 160.

On or about August 17, 1995, I continued the investigation of the stability of thermostable polymerases by comparing the activities of samples of *Taq* polymerases which had been stored at -20° C and 4° C. This activity was recorded on page 73 of notebook 3903. A copy thereof is attached as Exhibit 161.

On or about August 17, 1995, Carolyn Combs, working under supervision by me, further characterized *Tne* polymerase by performing additional PCR reactions using DNase-treated *Tne* polymerase, and determined that KCl did not inhibit the smear formation. This activity was recorded on page 175 of notebook 4092. A copy thereof is attached as Exhibit 162.

On or about August 18, 1995, Carolyn Combs, working under supervision by me, continued characterizing *Tne* polymerase. She performed additional PCR reactions using DNase treated *Tne* polymerase. This activity was recorded on pages 176-177 of notebook 4092. A copy thereof is attached as Exhibit 163.

On or about August 21, 1995, I further characterized the activity of the exonuclease mutant of Klenow fragment by performing strand displacement assays on the exo$^-$ mutant of Klenow polymerase. This activity was recorded on page 74 of notebook 3903. A copy thereof is attached as Exhibit 164.

On or about August 21, 1995, Carolyn Combs and I optimized the Mg^{++} concentration in PCR reactions with *Tne* polymerase. Additionally, they attempted to see if DNase- treated *Tne* will inhibit untreated *Tne* to determine what effect of DNase treatment has on *Tne* activity. This activity was recorded on pages 179-181 of notebook 4092. A copy thereof is attached as Exhibit 165.

On or about August 22, 1995, Carolyn Combs, working under supervision by me, repeated the attempt to determine if DNase- treated *Tne* polymerase would inhibit untreated *Tne* polymerase. This activity was recorded on pages 182-187 of notebook 4092. A copy thereof is attached as Exhibit 166.

On or about August 23, 1995, Carolyn Combs, working under supervision by me, analyzed results from experiments involving the smear on the gels of the products of the *Tne* PCR reaction, and conclusions were discussed. This activity was recorded on page 184 of notebook 4092. A copy thereof is attached as Exhibit 167.

On or about August 24, 1995, I continued attempts to determine the cause of the smear on gels from *Tne* PCR reactions. This activity was recorded on page 75 of notebook 3903. A copy thereof is attached as Exhibit 168.

On or about August 28, 1995, I repeated my attempt at incorporating ^{32}P into the smear from the *Tne* PCR reactions. A new sample of *Tne* polymerase was used. This activity was recorded on page 82 of notebook 3903. A copy thereof is attached as Exhibit 169.

On or about August 29, 1995, I began characterization of the new *Tne* FY mutant. I performed processivity assays on the *Tne* FY mutant as well as UlTma® polymerase and Elizabeth Flynn's preparation of 3'-5' exo$^-$ mutant *Tne* polymerase (*see* Exhibits F-3 through F-18). This activity was recorded on page 86 of notebook 3903. A copy thereof is attached as Exhibit 170.

On or about August 31, 1995, I began characterizing *Tne*Δ5FY and comparing its activity to that of other thermostable polymerases. In this experiment I assayed *Taq*, Vent® and *Tne*Δ5FY mutant for the ability to elongate and incorporate thymidines into a new DNA strand from a uracicontaining template. Additionally, I outlined conditions for 3' exonuclease assays on wild-type *Tne*, UlTma® and the *Tne*Δ5FY mutant. This activity was recorded on page 88 of notebook 3903. A copy thereof is attached as Exhibit 171.

On or about September 1, 1995, I further characterized the activity of the *Tne*Δ5FY mutant. I performed 3' exonuclease assays on wild-type *Tne*, UlTma® and the *Tne*Δ5FY

polymerases. Additionally, I attempted PCR reactions with wild-type *Tne* and the *Tne*Δ5FY mutant to optimize buffer conditions. This activity is recorded on pages 92-93 of notebook 3903. A copy thereof is attached as Exhibit 172.

On or about September 6, 1995, I investigated the use of the *Tne*Δ5FY mutant in PCR reactions. I optimized conditions for PCR with *Tne*Δ5FY at varying template lengths and buffer conditions. This activity was recorded on pages 94-95 of notebook 3903. A copy thereof is attached as Exhibit 173.

On or about September 7, 1995, I continued optimizing PCR conditions with *Tne*Δ5FY mutant. Specifically, salt conditions were optimized. This activity was recorded on pages 96-97 of notebook 3903. A copy thereof is attached as Exhibit 174.

On or about September 9, 1995, I prepared *Tth* polymerase for shipment. This activity was recorded on page 98 of notebook 3903. A copy thereof is attached as Exhibit 175.

5. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or document or any registration resulting therefrom.

Further, declarant sayeth not.

Date: 11-24-01

Name: Roger Lasken

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PUBLICATIONS

Lasken, R. S. and Goodman, M.F. The biochemical basis of 5-bromouracil-induced mutagenesis: heteroduplex base mispairs involving bromouracil in GC to AT and AT to GC mutational pathways. J. Biol. Chem. 259 (1984) 11491-11495.

Goodman, M.F., Hopkins, R., **Lasken, R. S.** and Mhasker, D.N. The biochemical basis of 5-bromouracil- and 2-aminopurine-induced mutagenesis. In: Genetic Consequences of Nucleotide Pool Imbalance--Environmental Science Research (F.J. deSerres,ed.), Plenum Press, New York (1984),pp. 409-423.

Lasken,R. S. and Goodman, M.F. A fidelity assay using "dideoxy" sequencing: measurement of sequence dependence and frequency of forming 5-bromouracil-guanine base mispairs. Proc. Natl. Acad. Sci. USA 82 (1985) 1301-1305.

Lasken, R. S. and Kornberg, A. The beta subunit dissociates readily from the *Escherichia coli* DNA polymerase III holoenzyme. J. Biol. Chem. 262 (1987) 1720-1724.

Maki, M., Maki, S., **Lasken, R. S.** and Kornberg, A. DNA polymerase III holoenzyme: subunits and functions. In: DNA Replication and Recombination, (R. McMacken and T. Kelly, eds.), Alan R. Liss, Inc., New York (1987), pp. 63-73.

Kornberg, A., Baker, A.T., Bertsch, L.L., Bramhill, D., Funnell, B.E., **Lasken, R.S.**, Maki, H., Maki, S., Sekimizu, K. and Wahle, E. Enzymatic studies of replication of oriC plasmids. In: DNA Replication and Recombination, (R. McMacken and T. Kelly, eds.), Alan R. Liss, Inc., New York (1987), pp. 137-149.
Lasken, R. S. and Kornberg, A. The n' protein of *Escherichia coli* is a DNA helicase. J Biol. Chem. 263 (1988) 5512-5518.

Wahle, E., **Lasken, R. S.** and Kornberg, A. The dnaB-dnaC protein complex of *Escherichia coli*. I. Formation and properties. J. Biol. Chem. 264 (1989) 2463-2468.

Wahle, E., **Lasken, R. S.** and Kornberg, A. The dnaB-dnaC protein complex of *Escherichia coli*. II. Role of the complex in mobilizing dnaB function. J. Biol. Chem. 264 (1989) 2469-2475.

Lasken, R. S., Schuster, D., and Rashtchian, A. Archaebacterial DNA polymerases tightly bind uracil-containing DNA. J. Biol. Chem. 271 (1996) 17692-17696.

Lasken, R. S. Review article. The proliferating cell nuclear antigen: The mechanism to replicate chromosomes and respond to DNA damage. Current Oncology (1998) vol. 5, number 3.

Zhao Y, Jeruzalmi D, Moarefi I, Leighton L, **Lasken R**, Kuriyan J. Crystal structure of an archaebacterial DNA polymerase. Structure Fold Des 1999 Oct 15;7(10):1189-99

G. Nallur, C. Luo, L. Fang, S. Cooley, V. Dave, J. Lambert, K. Kukanskis, Stephen Kingsmore, and **R. S. Lasken**, B. Schweitzer. Signal Amplification by Rolling Circle Amplification on DNA Microarrays. In Press Nucleic Acids Res.

Senior Authorships at MSI

Frank B. Dean, J. Nelson, T. Giesler, and **R. S. Lasken**. Rapid Amplification of Plasmid and Phage DNA Using Phi29 DNA Polymerase and Multiply-Primed Rolling Circle Amplification. Genome Research, in Press.

A. Faruqi, S. Hosono, M. Driscoll, F. Dean, O. Alsmadi, R. Bandaru, G. Kumar, B. Grimwade, Q. Zong, Z. Sun, Y. Du, S. Kingsmore, T. Knott, and **R. S. Lasken**. High-Throughput Genotyping of Single Nucleotide Polymorphisms with Rolling Circle Amplification. Manuscript in preparation

F. Dean, A. F.Faruqi, S. Hosono, M.Driscoll, R. Bandaru, X. Wu, Q. Zong, **R. S. Lasken**. Whole Genome Rolling Circle Amplification. Manuscript in preparation

Representative Conference abstracts

Cornell University Medical College

Society For Neuroscience, St. Louis, 1990.
R.S. Lasken, L. Lyandvert, D.J. Reese, Single-Stranded DNA Binding Protein is Detected in Mitotic and Postmitotic Embryonic Rat Neurons.

Life Technologies, Inc.

Human Genome Conference, Santa Fe, New Mexico, 1991.
R.S. Lasken, A. Haces, G. Gebeyehu, DNA Polymerase Specificity for Rhodamine, Fluorescein, and Biotin Labeled Nucleotides.

PolyGenomics, Inc.

6th World Congress on Psychiatric Genetics, Bonn, Germany, 1989. Lasken, R., Sirugo, G., Ward, D., and Weissman, S.
Advances in Gene Mapping Technologies: SNPs, Chips, and Rolling Circles.

Technical Writing

Life Technologies Inc., Taq DNA Polymerase Technical Bulletin 18038-1

Patents

US 09/398,215. Methods for identifying DNA sequences for use in comparing DNA samples by their lack of polymorphism. Inventers: Sherman Weissman and **Roger Lasken**

US 6,287,825 B1. Methods for reducing the complexity of DNA sequences. Inventers: Sherman Weissman and **Roger Lasken**

US 6,235,502 B1. Methods for selectively isolating DNA using rolling circle amplification. Inventers: Sherman Weissman and **Roger Lasken**

Serial No: 09/605,192. Claims allowed. Multiply-primed amplification of nucleic acid sequences.

Inventers: **Roger Lasken**, Frank Dean, John Nelson

Multiple displacement amplification. Filed 2001. Inventers: Frank Dean, **Roger Lasken**, et. al.